Effect of Lactic Acid Administration in the Drinking Water During Preslaughter Feed Withdrawal on *Salmonella* and *Campylobacter* Contamination of Broilers[2]

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ABSTRACT The crop is a known source of *Salmonella* and *Campylobacter* contamination. We evaluated the use of selected organic acids (0.5% acetic, lactic, or formic) in drinking water during a simulated 8-h pretransport feed withdrawal (FW). *Salmonella typhimurium* was recovered from 53/100 control crops and from 45/100 of crops from acetic acid-treated broilers. However, treatment with lactic acid (31/100) or formic acid (28/76) caused significant ($P < 0.05$) reduction in incidence. Reductions of recovered incidence were also associated with reduced numbers of *S. typhimurium* recovered (e.g., control, log 1.45 cfu/crop; lactic acid, 0.79 cfu/crop). In an additional commercial farm study, broilers were provided 0.44% lactic acid during a 10-h FW (4 h on the farm and 6 h transport) and pre-FW crop, post-FW crop, and pre-chill carcass wash samples were collected for *Campylobacter* and *Salmonella* detection. Crop contamination with *Salmonella* was significantly reduced by lactic acid treatment (6/175) as compared with controls (29/175). Importantly, *Salmonella* isolation incidence in prechill carcass rinses was significantly reduced by 52.4% with the use of lactic acid (26/175 vs. 55/176). Crop contamination with *Campylobacter* was significantly reduced by lactic acid treatment (62.3%) as compared with the controls (85.1%). Lactic acid also reduced the incidence of *Campylobacter* found on pre-chill carcass rinses by 14.7% compared with the controls. These studies suggest that incorporation of lactic acid in the drinking water during pretransport FW may reduce *Salmonella* and *Campylobacter* contamination of crops and broiler carcasses at processing.

(*Key words*: *Campylobacter*, chicken, crop, lactic acid, *Salmonella*)

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INTRODUCTION

A standard management practice in commercial broiler production is the removal of feed immediately prior to transportation to slaughter and processing. The purpose of feed withdrawal (FW) is to enhance the clearance of the gastrointestinal tract and to reduce contamination of poultry carcasses (May and Lott, 1990). However, the antemortem practice of FW has been shown to increase the number of *Salmonella*- and *Campylobacter*-contaminated crops. Ramirez and co-workers (1997) found that FW increased the incidence of *Salmonella* isolation from crops of experimentally and naturally infected broilers. Similarly, FW increased the incidence of *Campylobacter* in the crops of commercially reared broilers (Byrd et al., 1998). The results of these studies suggest that crop contamination during the preslaughter feed withdrawal period represents a significant critical control point for reducing entry of *Salmonella* and *Campylobacter* into the processing plant. The normal microflora of the crop has been shown to compete with coliform and pathogenic bacteria. Part of the resident population of bacteria includes *Lactobacilli*, a major producer of lactic acid (Fuller, 1973, 1977; Fuller and Brooker, 1974; Humphrey et al., 1993). Feed withdrawal has been reported to cause an increase in broiler crop pH and a decrease in lactic acid concentrations (Humphrey et al., 1993; Corrier et al., 1999b). The

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Abbreviation Key: BGA = brilliant green agar; FW = feed withdrawal; LA = lactic acid; NA = nalidixic acid; NO = novobiocin.

decrease in crop lactic acid and increased pH may provide an improved environment for the proliferation of pathogenic bacteria. Crop contents from hens deprived of feed have been shown to increase the virulence of *Salmonella* by increasing the expression of the genes necessary for intestinal invasion in vitro (Durant et al., 1999).

We evaluated the use of selected concentrations of several organic acids (acetic, lactic, or formic) in the drinking water during simulated pretransport FW.

MATERIALS AND METHODS

Experimental Design

Experiment 1. The effect of several organic acids (0.5% acetic, lactic, or formic) on crop pH and *Salmonella* contamination of crop contents was evaluated in five experimental broiler flocks. For each flock, 5-wk-old broilers were divided into four groups of 20 birds each and were placed in floor pens on new pine shavings. Free access to water and a balanced unmedicated corn-soybean meal based ration was provided to birds acclimated for 1 wk prior to introduction of the experiment.

During an 8-h FW, broilers were provided distilled water, 0.5% acetic acid, 0.5% lactic acid, or 0.5% formic acid. Each bird was challenged with 10^8 cfu novobiocin (NO)- and nalidixic acid (NA)-resistant *S. typhimurium* upon arrival (Day 35) and again 1 d prior to termination of the experiment (Day 41). Crops and ceca were collected aseptically from 20 broilers from each group after an 8-h FW (Day 42). Ten of the 20 broilers were selected randomly, and the crop pH was determined by insertion of a glass pH electrode[3] through an incision in the crop wall to ensure that the electrode remained in contact with the crop mucosal surface. The crop and a cecum from each sampled broiler were incised aseptically and placed in separate Whirl-pac™ bags.[4] Ten milliliters of sterile water was added to each bag, and the sample was stomached for 30 s.[5] The blended crop samples were cultured for NO- and NA-resistant *S. typhimurium* by selective enrichment procedures, serial dilution, and spread plating described below. Similarly, blended ceca samples were cultured as described above.

Challenge inoculum for Experiment 1 was prepared from a primary poultry isolate of *S. typhimurium* obtained from the National Veterinary Services Laboratory, Ames, Iowa. The isolate was selected for resistance to NO and NA and was maintained in media containing 20 μg/mL NA and 25 μg/mL NO. Challenge inocula were prepared from an overnight soy broth culture serially diluted in sterile phosphate buffered saline. The optical density of the cell dilution was measured with a spectrophotometer[6] at 625 nm, and the number of cells for each inoculum was determined using a standard curve. The viable cell concentrations of the challenge inocula were confirmed by colony counts on brilliant green agar (BGA) plates.[7]

Experiment 2. The effects of 0.44% lactic acid (LA) on crop pH and *Salmonella* contamination of crop contents were evaluated in commercial broiler flocks. Seven randomly selected farms were evaluated for the detection of salmonellae by using double-strength skim milk drag-swabs (Kingston, 1981; Mallinson et al., 1989). Two houses on each farm were selected by matching similar drag-swab contamination numbers. The broiler houses were paired by the number of *Salmonella*-positive drag-swabs found in each of the houses evaluated (i.e., houses with three of four *Salmonella*-positive first tests each were paired). Fourteen commercial broiler houses that were *Salmonella* positive by drag-swabs were selected, and broilers from each house were provided food-grade LA or nontreated water (control) during FW.[8] The broiler houses were paired by the number of *Salmonella*-positive drag-swabs found in each of the houses evaluated. Food-grade LA was obtained from a commercial producer at purity of 88% LA and was immediately diluted prior to administration with an equal volume of sterile, double-distilled water. The food-grade LA (44%) was diluted 1:100 with the local water source by using a commercial medicator to provide a final concentration of 0.44% LA in the nipple drinker water lines.[9] Nipple drinker water lines were flushed with 0.44% LA until acidity (pH ~3.00) was detected at the end of each water line. The LA water was provided approximately 1 h prior to FW and continued until transport to the processing plant. Crops were collected aseptically from 25 randomly selected market-age broilers from each of 14 commercial rearing house flocks before preslaughter feed removal (full-fed controls) and from another 25 randomly selected broilers removed from the processing line after inspection by the USDA-Food Safety Inspection Service inspectors. Each of the crops was incised aseptically, and the tissues and contents were placed in a plastic bag, maintained in an ice chest near 4 C, and transported to the laboratory. All crop samples were cultured by selective enrichment for salmonellae within 24 h after collection as described below.

Twenty-five carcasses were selected randomly after the final wash prior to entering the chill tank for carcass rinse. Each carcass was removed from the processing line using a clean glove and placed in an individual polyethylene bag.[10] One hundred milliliters of sterile water was aseptically added to each carcass, the bag was sealed with cable ties, and the content was vigorously shaken by hand for 1 min. The rinse fluid was collected in sterile sealable containers and held on wet ice for transport to our laboratory (18 h).

Salmonella and Campylobacter Culture Procedures

Thirty milliliters tetrathionate broth was added to each plastic bag containing crops collected from the broilers

[3]Cole Parmer Model 05669-20, Niles, IL 60714.
[4]Nasco, Fort Atkinson, WI 53538.
[5]Teckmar Stomacher 80, Laboratory Blender, Cincinnati, OH 45222.
[6]Spec 20D, Milton Roy, Analytical Products Division, Rochester, NY 14625.
[7]Oxoid, Unipath Ltd., Basingstoke, Hampshire, England RG24 8PW.
[8]Purac America, Chicago, IL 60069.
[9]Model #7 GPM, Dosatron International Inc., Clearwater, FL 34625.
[10]63.5 cm × 88.3 cm, Fisher Scientific, Pittsburgh, PA 15275.

at commercial broiler houses.[11] The crop samples were blended for 30 s in a stomacher and incubated for 24 h at 37 C. After incubation, the broth was streaked onto BGA plates, incubated for an additional 24 h at 37 C, and examined for suspect *Salmonella* colonies. Suspect colonies were confirmed biochemically on triple sugar iron agar and lysine iron agar by previously reported procedures (Andrews et al., 1992).

Crop samples collected during Experiments 1 and 2 were cultured by selective enrichment and by plating serial dilutions. One milliliter of the blended crop contents was transferred into 10 mL of tetrathionate broth and incubated for 24 h at 37 C. After incubation, the broth was streaked onto NA/NO-BGA plates, and the presence of *Salmonella* was confirmed biochemically as described above. In Experiment 1, an additional 1.0 mL sample of the blended crop contents was serially diluted through three tubes containing 9 mL each of sterile Butterfield's buffer (1:10, 1:100, and 1:1,000 dilutions). A portion (0.10 mL) was removed from the undiluted crop contents and from each dilution tube and spread-plated onto NA/NO-BGA plates to produce final dilutions of 1:10, 1:100, 1:1,000, and 1:10,000. The plates were incubated for 24 h at 37 C, and *S. typhimurium* colony-forming units were enumerated. For culture of the carcass rinse samples for *Salmonella* in Experiment 2, an equal volume of concentrated (2x) tetrathionate broth base[12] was added to the rinse fluid of each sample. Enriched samples were incubated at 37 C for 24 h and streaked for isolation on BGA containing NO. After 24 h incubation, a suspect colony from each plate was confirmed biochemically (Andrews et al., 1992).

Prior to tetrathionate enrichment of crop and carcass rinse samples for Experiment 2, 2 mL of the blended crop material or carcass rinse was transferred to 20 mL of modified Bolton's broth and incubated for 4 h at 37 C, followed by 20 h at 42 C in a microaerobic environment (5% O_2, 10% CO_2, and 85% N_2). After enrichment, samples were streaked for isolation on Campy-ceflex plates and incubated for 24 to 48 h at 42 C in the microaerobic environment (Stern et al., 1992). Suspect *Campylobacter* colonies were confirmed serologically using a latex-agglutination kit[13] specific for *Campylobacter jejuni*, *Campylobacter coli*, and *Campylobacter lari*.

Statistical Analysis

In Experiment 1, all five replicates were combined, and differences among groups in crop pH and $\log_{10}$ cfu *Salmonella* were determined by one-way ANOVA (Snedecor and Cochran, 1967) using the general linear model procedure in the SAS® statistical software (SAS Institute, 1987). Variable means for treatments showing significant differences in the ANOVA were further separated using Duncan's multiple-range test. *Salmonella* colonies were logarithmically transformed prior to analysis to achieve homogeneity of variance and were expressed as log10 cfu. In Experiments 1 and 2, differences among groups in the incidence of *Salmonella* crop contamination and incidence of *Salmonella*-positive carcass rinses were analyzed by chi-square analysis. In Experiment 2, data were compared between paired control houses and the corresponding treatment house and between total control houses and total treated houses. All analyses were conducted with commercial statistical analysis software (Luginbuke and Schlotzhauer, 1987). All statements of significance are based on $P < 0.05$ unless noted otherwise.

RESULTS

Experiment 1

In broilers given selected organic acids (0.5% acetic, lactic, or formic) in the drinking water during an 8-h FW period, crop pH and number of *Salmonella* colony-forming units recovered from ceca of birds fed LA were not statistically ($P < 0.05$) different from controls (Table 1). The incidence of *Salmonella* culture-positive crops decreased significantly after the administration of 0.5% LA or 0.5% formic acid, but not 0.5% acetic acid, in the drinking water during FW as compared with the controls. No significant differences were observed in the incidence of *Salmonella* culture-positive ceca after administration of the organic acids.

Experiment 2

Lactic acid (0.44%) provided in the drinking water of broilers during a 10 to 14 h FW period significantly reduced the incidence of *Salmonella* culture-positive post-FW crops and prechill carcass rinsates in House 4 and total samples as compared with the nontreated controls (Table 2). In addition, LA significantly reduced the incidence of *Salmonella* culture-positive, prechill, carcass rinsate in House 2 as compared with the nontreated controls. Lactic acid provided in the drinking water significantly reduced the incidence of *Campylobacter* culture-positive crops (before and after FW) and in prechill carcass rinsates in the combined flocks as compared with the nontreated controls (Table 3). In Houses 4, 6, and 10, lactic acid significantly ($P < 0.005$) reduced the incidence of *Campylobacter* culture-positive, post-FW crops as compared with the nontreated controls. In Houses 8 and 10, pretreatment and pre-FW crops had significantly higher or lower incidence of *Campylobacter*-positive crops when compared with the pre-FW control crops, respectively. Broilers provided LA in the drinking water had significantly lower incidence of *Campylobacter* culture-positive, prechill carcass rinsate in House 10 as compared with the nontreated controls. Consumption of water was recorded for four houses (two treated and two control), and no significant differences were observed between treated and control houses (data not shown).

[11]Difco Laboratories, Detroit, MI 48232.
[12]Becton-Dickinson, Cockeyville, MD 21030.
[13]Integrated Diagnostics, Inc., Baltimore, MD 21227.

TABLE 1. Effect of selected acids on *Salmonella typhimurium* crop and cecal colonization in broilers (five trials)

Group	Log_{10} *Salmonella*/g crop contents	Crop culture positive/total (%)	Crop pH (n = 40)	Log_{10} *Salmonella*/g ceca contents	Ceca culture positive/total (%)
Control	1.45 + 1.52[a]	53/100 (53%)	5.77 + 0.82[a]	2.33 + 1.98[a]	53/80 (66.3%)
Acetic acid (0.5%)	0.96 + 1.35[b]	45/100 (45%)	5.34 + 0.75[b]	1.70 + 1.49[b]	49/80 (61.3%)
Lactic acid (0.5%)	0.79 + 1.30[b]	31/100 (31%)*	4.79 + 0.87[c]	1.93 + 1.78[ab]	47/80 (58.8%)
Formic acid (0.5%)	0.94 + 1.36[b]	28/76 (36.8%)*	4.80 + 1.00[c]	1.73 + 1.40[b]	31/60 (51.7%)

[a–c]Mean values within the same column with no common superscripts differ significantly ($P \leq 0.05$).

*A significant ($P \leq 0.05$) difference was found in the number of *Salmonella*-positive crops between treatment and controls.

DISCUSSION

Withholding feed from broilers 8 to 12 h prior to processing allows partial clearance of the gastrointestinal tract and reduces fecal contamination of poultry carcasses (May and Lott, 1990). Although the number of total bacteria may decrease, the conditions of the gastrointestinal tract may favor pathogen growth. Feed withdrawal increases the survival of *Salmonella* in laying hens (Durant et al., 1999) and in the bovine rumen (Brownlie and Grau, 1967). Similarly, feed-deprived broilers have significantly higher *Salmonella* and *Campylobacter*-positive crop contamination rates compared with fully fed controls (Ramirez et al., 1997; Byrd et al., 1998; Corrier et al., 1999a).

Increased *Salmonella* and *Campylobacter* crop contamination may be partially explained by increased consumption of contaminated litter during FW (Corrier et al., 1999a). Feed withdrawal and apparent hunger due to FW increases the peristaltic activity of the crop and increases the emptying of the crop (Sturkie, 1976). Thus, the increased load of pathogenic bacteria transported through the gastrointestinal tract may increase the number of pathogens in the lower gastrointestinal tract. Feed deprivation has also been shown to increase crop pH, decrease *Lactobacillus* numbers, and decrease LA concentration in the crop, causing a more favorable environment for pathogenic bacteria (Humphrey et al., 1993; Corrier et al., 1999b; Durant et al., 1999). Thus, as conditions that favor pathogenic bacterial colonization are combined with increased pathogen ingestion, it is perhaps not surprising that the overall incidence of crop contamination may increase.

The use of acetic, lactic, or formic acid in the drinking water of market-age broilers significantly reduced crop pH and decreased the recovery of *Salmonella* from crop samples. One drawback reported in this study was that acetic, lactic, and formic acids (30, 44.9, and 31.7%, respectively) reduced water consumption during the 8-h FW period when compared with that of the controls. Because of the reduced water consumption and to comply with producers demand for an easy application of the product,

TABLE 2. Effect of 0.44% lactic acid (LA) provided during feed withdrawal (FW) on *Salmonella* colonization of the crop and carcass in market-age broiler chickens

Farm	House	Treatment	Total FW (time on water treatment) (h)	*Salmonella*-positive (Positive samples/total samples)		
				Pre-FW crop[1]	Post-FW crop[2]	Prechill carcass wash[3]
1	1	Control	10 (4)	3/25	0/25	5/25
	2	LA	10 (4)	1/25	2/25	0/25*
2	3	Control	10 (4)	9/25	23/25	23/26
	4	LA	10 (4)	5/25	0/25***	3/25***
3	5	Control	12 (8)	0/25	4/25	8/25
	6	LA	12 (8)	2/25	2/25	13/25
4	7	Control	10 (8)	1/25	0/25	4/25
	8	LA	10 (8)	2/25	0/25	1/25
5	9	Control	11 (8)	5/25	0/25	5/25
	10	LA	11 (8)	0/25*	0/25	4/25
6	11	Control	14 (12)	3/25	1/25	9/25
	12	LA	14 (12)	3/25	1/25	4/25
7	13	Control	11 (9)	1/25	1/25	1/25
	14	LA	11 (9)	1/25	1/25	1/25
	Total	Control		22/175 (12.6%)	29/175 (16.6%)	55/176 (31.3%)
	Total	LA		14/175 (8.0%)	6/175 (3.4%)***	26/175 (14.9%)***

[1]Crops were removed from broilers prior to feed withdrawal.

[2]Crops were removed from carcasses after evisceration (before final wash).

[3]Carcass washes were performed immediately after the final wash but before chilling. A significant difference was found between the number of positive controls and positive, treated crops (*$P \leq 0.05$; ***$P \leq 0.001$).

TABLE 3. Effect of 0.44% lactic acid (LA) provided during feed withdrawal (FW) on *Campylobacter* colonization of the crop and carcass in market-age broiler chickens

Farm	House	Treatment	Total FW (time on water treatment) (h)	*Campylobacter*-positive (Positive samples/total samples)		
				Pre-FW crop[1]	Post-FW crop[2]	Prechill carcass wash[3]
1	1	Control	10 (4)	0/25	5/25	14/25
	2	LA	10 (4)	0/25	8/25	8/24
2	3	Control	10 (4)	7/25	24/25	24/25
	4	LA	10 (4)	6/25	16/25**	20/25
3	5	Control	12 (8)	7/25	23/25	21/25
	6	LA	12 (8)	11/25	14/25**	23/25
4	7	Control	10 (8)	15/25	24/25	19/24
	8	LA	10 (8)	23/25*	20/25	17/25
5	9	Control	11 (8)	22/25	24/25	22/25
	10	LA	11 (8)	10/25***	8/25***	11/25**
6	11	Control	14 (12)	18/25	24/25	25/25
	12	LA	14 (12)	23/25	21/25	25/25
7	13	Control	11 (9)	21/25	25/25	25/25
	14	LA	11 (9)	24/25	22/25	24/25
	Total	Control		90/175	149/175 (85.1%)	150/175 (85.7%)
	Total	LA		97/175	109/175 (62.3%)***	128/175 (73.1%)**

[1]Crops were removed from broilers prior to feed withdrawal.

[2]Crops were removed from carcasses after evisceration (before final wash).

[3]Carcass washes were performed immediately after the final wash but before chilling. A significant difference was found between the number of control and treated, positive crops (*$P \leq 0.05$; **$P \leq 0.005$; ***$P \leq 0.001$).

0.44% LA was used on the commercial farm studies instead of the experimental concentration of 0.5% LA.

In the commercial farm study, broilers were provided LA (0.44%) in the drinking water during a 10 to 14 h FW, and then pre-FW crop, post-FW crop, and prechill carcass wash samples were collected for *Campylobacter* and *Salmonella* detection. Postharvest crop contamination with *Salmonella* or *Campylobacter* was significantly reduced by 0.44% LA treatment as compared with controls. More importantly, *Salmonella* and *Campylobacter* isolation in prechill carcass rinses were significantly reduced by 52.4 and 14.7% LA, respectively. Consumption of water was recorded for four houses (two treated and two control), and no significant differences were observed between treated and control houses. Although not evaluated in this study, carcass yields were not different as reported by two commercial broiler producers.

Salmonella results seemed to vary with each house, and most of the overall effect came from a few houses for *Salmonella* (Table 2: Houses 1 to 4). One possible explanation for these results was that each carcass rinsate sample was detected to be *Salmonella* positive or negative and did not account for possible reductions in the number of *Salmonella* that may have occurred with the LA treatment. Furthermore, reexamination of the prechill carcass rinsate data without Houses 1 to 4 revealed that broilers provided LA in the drinking water had significantly lower *Salmonella* recovery numbers (23/125; 13.1%) compared with the controls (27/125; 21.6%).

These studies suggest that incorporation of some organic acids in the drinking water during pretransport FW may reduce *Salmonella* and *Campylobacter* contamination of crops and broiler carcasses at processing. The LA provided in the drinking water reduces the pH of the crop and may provide a temporary carbon source for bacteria normally present in the crop. Several conditions that naturally occur during FW were reversed with the addition of organic acids in the drinking water of market-age broilers.

Government regulations (Pathogen Reduction Act) have brought a need for cost-efficient approaches to reduce food-borne pathogens without dramatically altering present management techniques. The results of the present study suggest a possible means to reduce food-borne pathogens that can be incorporated into existing commercial management procedures.

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